DISCUSSION

Following Reading of Lindegren's and Tatum and Beadle's Papers

Dr. Demerec: Yesterday I attended a seminar at the University of Missouri where Dr. Briggs from the University of California discussed the disease resistance of plants. He described four genes which determine resistance to smut in wheat and three out of those four are located in one chromosome. Now there are several instances where genes of like action are located in the same chromosome, and we have a number of cases where genes of very similar action are located very close together. I wonder if Dr. Beadle has any indication of instances where genes closely similar are located close together.

Dr. Beadle: There are a number of cases where they seem to be associated in ways that you might not expect. For example, there are two different albino strains that appear to be genetically different, yet they are so close together they practically never cross over. There is one chromosome, you may remember, concerned with three pyrimidineless mutants and I would hesitate to say whether they are chance distributions or not. You cannot take the chromosome map as a random sample. By our procedure we tend to pick up a non-random assortment of linkages.

Question: In some cases several genes may be involved in one very simple step of the over-all synthesis. That seems to indicate that those genes work together in the manufacture of the enzyme for that step. Is there any evidence that some other substance may be supplied which would enter into the formation of the enzyme?

Dr. Tatum: We have no evidence on that question. In the cases illustrated in which there are four mutations concerning the synthesis of arginine there must be four different steps involved.

Dr. Muller: Where you have two or more different mutations affecting the same synthesis and the substances are diffusible can you always get growth if you give the product of one of them to the other?

Dr. Beadle: Yes, if the substances are stable.

Dr. Stadler: Once you find the essential substance that is lacking how do you keep the culture growing?

Dr. Tatum: On a minimum amount; the cultures are given enough to grow, but no excess.

Dr. Cori: These intermediate products accumulate. To have them accumulate you stop formation of the final product. Why should the reaction continue in the intermediary stages if the final product is supplied?

Dr. Beadle: You don't have intermediates formed if you give a sufficient

amount of the final product, just enough to tease it a little. If you give plenty of adenine no pigment whatever is found, but if you give it just the right minimal amount, a large amount of purple pigment is formed.

DR. GODDARD: Dr. Tatum, how long does it take to bring about an adaptation that you can detect, a matter of hours, days, or longer?

DR. TATUM: Ordinarily, it is a matter of days, not hours.

DR. GODDARD: Is there any relation between the amount of mycelium and adaptation time?

DR. TATUM: I think there is; probably larger amounts of mycelium adapt more rapidly.

DR. GODDARD: I think there is a conceivable biochemical change important in adaptation and related to the volume of the tissue versus volume of the liquid. If you started from A and went to Z and had no intermediates, it would take an extraordinarily long time for anything to accumulate; time measured in terms of hours and not minutes. If, in a mutant, you suddenly have blocked one stage it might take a good many hours to build up sufficient concentration so a new reaction can start. This type of adaptation could not involve changes in existing enzymes or genes, but a real change in components. As James Franck pointed out, concerning a reaction which starts within a minute or so after illumination occurs, if all the intermediates between CO_2 and carbohydrates had to be built up it would take eight hours. As a consequence, one knows the intermediates must be present.

DR. DELBRÜCK: Can you tell us something about reverse mutations: whether they occur and can be recognized?

DR. BEADLE: The main difficulty in answering your question is the experimental difficulty in telling the difference between contamination and reverse mutation unless you have a special set-up because, of course, you would expect to get a wild type in reversion and you could also expect contamination by wild type.

DR. DELBRÜCK: It seems to me if this mutant can revert it should have a well-defined reverse mutation rate and should be distinguishable from an erratic contamination.

DR. BEADLE: If the mutation rate is high enough.

DR. DELBRÜCK: For spontaneous reverse mutation one could use Roepke's method of growing the mutants in the presence of minimal amounts of the missing factor and then see whether you finally get rapid growth.

DR. BEADLE: We have not done that extensively.

DR. HOLLAENDER: Have you found any difference between spontaneous mutation rates and rates produced by different types of radiation?

DR. BEADLE: We have not studied spontaneous mutation rate because, so far, we have not concerned ourselves with problems concerning mutation rate in

general. For one thing, we figured you could do a better job; also, we thought that the amount of testing we would have to do to determine the spontaneous mutation rate was so great we hesitated to undertake it.

DR. TATUM: I have a remark in connection with Dr. Goddard's suggestion. There is one mutant strain which requires thiazole and makes pyrimidine. This does not adapt to the slightest extent, no matter how long it is left. On the other hand, the thiaminless mutant strain which requires the intact molecule and which makes both intermediates adapts extraordinarily well. That might support this idea. However, in another instance involving pantothenic acid adaptation did not occur. So there are differences.

DR. GREENSTEIN: I would like to point out the very remarkable parallel between these effects that Dr. Tatum and Dr. Beadle have obtained and changes in neoplastic tissues. If one compares functions of normal living cells with those of tumors one finds similar reactions, but one must also assume that newer functions are induced. I wonder if Dr. Tatum and Dr. Beadle considered the possibility that mutant effects may have induced the formation of new functions, perhaps unnecessary, but present nevertheless.

DR. TATUM: I think there are pertinent examples of the formation of new substances in the production and utilization of intermediates and the production of pigments. It is perfectly possible that those are merely instances which we picked up; perhaps a whole series of new reactions are instigated by means of blocked reactions.

DR. MULLER: Would it be possible to test blocking any of the enzymes by anti-bodies?

DR. TATUM: I think that is quite possible to do. Perhaps Dr. Emerson has some comments.

DR. EMERSON: It would be easier to block with substrate; antibodies usually are not directed against the active groups on the enzymes.

DR. EDGAR ANDERSON: I would like to ask Dr. Beadle if he has information on chiasma frequencies.

DR. BEADLE: No, I haven't any, and I think Dr. McClintock has not made actual counts, although I think she could give you an estimate. I suppose the average is perhaps between one and two, possibly around two, but that is just a very rough guess.

DR. STADLER: I notice in the general discussion by Dr. Tatum that when there are, say, four genes affecting a step or sequence of steps, it is apparently assumed that it must involve four or more enzymes. I wonder if the results contradict the assumption that production of a single enzyme could be affected by two, three, or four genes?

DR. TATUM: We feel quite certain that there are a number of instances in which a number of genes will affect a common enzyme.

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FOLLOWING READING OF SPIEGELMAN'S PAPER

DR. GODDARD: It would be of some importance to get at the nature of the enzyme-substrate compound. Do you know the Michaelis constant? Is the amount of galactose tied up with galactozymase dependent upon the galactose concentration?

DR. SPIEGELMAN: In attempting to obtain rate-concentration curves we ran into several difficulties. At high concentrations there is an inhibition of adaptation, probably osmotic in nature. At low concentrations it is difficult to keep the concentrations constant due to the preadaptive aerobic utilization of the galactose.

DR. GODDARD: The low concentration experiments are critical. Perhaps one might place the culture in a collodion bag suspended in a large volume of the galactose solution.

DR. BEADLE: Considering the four positive spores which segregate from the first hybrid, have you been able to demonstrate any difference at all in the two types, or have you looked for such a difference?

DR. SPIEGELMAN: As a matter of fact, a great deal of effort was expended in trying to find such differences. None was detectable. I might mention in passing that we tried to see whether the rates of adaptations differed between a haploid carrying two mel+ genes and one carrying only one such gene. Here again no difference could be established.

DR. BEADLE: In this diagram you have presented of gene action is it possible to suppose that the gene is originally determining the specificity of "P" which in turn somehow acts as a pattern for "E"? An analogous situation might be the transformation of pepsinogen into pepsin.

DR. SPIEGELMAN: I see no objection against a supposition of this kind. It will be noted, however, that it grants even more than I ask, that even in the presence of the controlling gene there exists a step in the chain which is autocatalytic and completely free of primary gene action.

DR. MULLER: I should like to raise the question of the specificity of the synthesis of "E", especially with relation to the gene. How likely is it to depend on factors other than this gene and "P"?

DR. SPIEGELMAN: You mean besides the mel+ gene?

DR. MULLER: Yes, other than the gene whose absence deprives it of the adaptation possibility.

DR. STADLER: I think the point mentioned by Dr. Muller is the one with which we are all concerned. It seems to me that the crucial question which must be considered here is whether we must, as a consequence of these experiments, add to our array of self-reproducing units a fundamentally new one; the self-reproducing enzyme molecule. Or, can we by not too complex or artificial a system construct a picture according to which we might account for the experiments without postulating a new self-reproducing unit? I know that Dr. Spiegel-

man has considered this question and I should like to ask him what assumptions he thinks are necessary to make it possible to explain the experimental results on a genic basis.

DR. SPIEGELMAN: We may assume the existence of a gene "X", different from either of the two mel+ genes already mentioned and capable, under certain conditions, of performing the same function. We must, in addition, ascribe the following properties to the "X" gene: it can only perform its function if kept in continuous contact with the enzyme. Thus the "X" gene would require the presence of the mel+ gene in order to start functioning. Once they were separated the "X" gene would remain functional only so long as sufficient enzymes were present in the cytoplasm; i. e., so long as the substrate was available. Another mechanism one might devise, although slightly more complicated, is that the gene "X" is stabilized directly by the substrate rather than by the enzyme. These are, I feel sure, not the simplest desirable mechanisms involving primary gene control. I am reasonably certain, however, that no mechanisms depending on genes will be able to avoid assuming that the functional stability of the controlling gene is dependent on either the substrate or the enzyme.

DR. GREENSTEIN: This hypothesis would seem to involve a serious revision of previously conceived notions of the nature of the gene. I wonder whether your reaction could not be modified somewhat by saying "P" plus "S" is equal to "E". In that way the character of your enzyme would be a function of your substrate.

DR. SPIEGELMAN: I don't see offhand that this is essentially different from the scheme I presented. The enzyme content is a function of substrate from the point of view of its stabilizing effect, and it is this aspect that I wished to emphasize. Evidence for a more direct involvement such as would be implied by the suggested modification is not available.

DR. GREENSTEIN: Are you certain that glucozymase is more stable than the galactozymase system?

DR. SPIEGELMAN: Yes. We have direct evidence in the form of both *in vitro* and *in vivo* comparisons.

DR. SONNEBORN: May I suggest that this other gene "X" we have been discussing seems to be excluded by the experiments in which removal of melibiose leads to loss of adaptability?

DR. SPIEGELMAN: The assumption of irreversible loss of function on removal of substrate (and enzyme) would explain the data.

DR. STADLER: It seems obvious that any other gene one would postulate would have to have some very artificial characters. The very nature of these characteristics could, by their artificiality, carry us a long way toward the necessity of assuming self-reproducing enzyme molecules. We may perhaps simplify the question under discussion if we stress the comparative plausibility of any proposed genic mechanism with that of the self-reproducing hypothesis.

DR. SONNEBORN: There is one difficulty with the genic mechanism. The "X" gene can apparently react to substrate when it is in the presence of the

mel+ gene but it loses that capacity when both the mel+ gene and the substrate are removed.

Dr. Spiegelman: If one is bent on retaining the gene mechanism it is not too difficult to get around this dilemma. One could assume that there is a sort of "position effect" in terms of a diffusible substance made by the mel+ gene required by the gene "X" for functional activity in the absence of substrate. Even simpler, it seems to me, would be to explain it in terms of interaction between gene "X" and enzyme, which latter we know is mediated by the mel+ gene.

Dr. Delbrück: Omitting for the moment the question of how one can avoid the self-duplication of enzymes, I should like to discuss another aspect of the problem. One property you have assumed which perhaps might at first glance appear peculiar is that the transformation from "P" to "E" can be catalyzed by two different agents, gene and enzyme. The first thing one would suspect is that perhaps "G" and "E" are very similar, a suspicion you have probably entertained. I might add that aside from these experiments and your formulation it is a suspicion one might have in any case, in view of the close correlation between gene and enzyme presented this morning by Dr. Beadle and Dr. Tatum. We would picture the present instance then in the following terms: you have a gene "G" which is stable so long as it remains in the nucleus. It produces an enzyme "E", similar to itself. When the enzyme gets into the cytoplasm it becomes unstable, a condition which, as you pointed out in the beginning of your talk, may be a very general one. Now if this unstable enzyme is stabilized by substrate then it can, in the cytoplasm, replace the gene. This seems to me a very fruitful picture. I do not, however, wish to lead you away from the search for alternative explanations.

Dr. Spiegelman: I should like to point out one difficulty I have encountered in trying to push the analysis along the lines suggested by Dr. Delbrück. If the genes and the enzymes they produce are similar why is it that the former are stable, whereas the latter are not? In terms of modern biochemical concepts the instability of the enzyme in the cytoplasm is the easier of the two to understand. All the components in the cytoplasm are in a state of flux and any given one can maintain itself only by balancing the rate of its disappearance with an equal rate of resynthesis. Here the substrate performs two functions, stabilizing the enzyme molecules and providing energy for synthetic activity. We have more or less direct evidence for this view. The rate of disappearance of enzyme in the absence of its substrate is proportional to the over-all metabolic turnover of the cells. Such unstable enzymes can be stabilized by depressing metabolic activity with, e. g., anaerobiosis or with sodium azide which prevents nitrogen assimilation. One way of explaining the stability of the gene on the same basis would be to assume that it is outside the metabolic cycle and that it alone of all protoplasmic units does not undergo continual breakdown and resynthesis. This raises the obvious difficulty of being forced, in a sense, to remove genes from the effects of

reactions which they control and with which they must necessarily be in intimate physiological contact.

DR. CORI: One fundamental question here is what is meant by reproduction of enzymes. What ideas do you have on this with respect to the mechanism and the nature of the precursor?

DR. SPIEGELMAN: I cannot go further at present than to say that the enzyme influences its own production. The details of the mechanism must await the elucidation of the nature of the precursor. With respect to the latter it seems to me unlikely that we are dealing here with synthesis all the way from the amino acids. It appears more likely that the precursor is an indifferent protein.

DR. BEADLE: I don't quite see the reason for the anxiety to avoid the concept of self-duplication of enzymes. In this connection, I should like to bring up again the pepsinogen-pepsin transformation and its relation to this problem.

DR. MULLER: I should differ with Dr. Beadle in just one respect. I think there is evidence here of self-reproduction; something akin to the gene. However, we do not know how general this phenomenon is. The concept is sufficiently novel to require more proof before its generalization is accepted. With respect to the pepsin formation it must be noted that it requires a very specific precursor, pepsinogen.

DR. BEADLE: How do we know that we do not have a specific precursor here much like pepsinogen? Wouldn't we then have what appears to be self-duplication in terms of transformation from precursor?

DR. GREENSTEIN: Pepsinogen is an inactive form which can be activated by either pepsin or hydrochloric acid.

DR. BEADLE: Couldn't "P" be an inactive form of "E"?

DR. GREENSTEIN: Perhaps, but it is an inactive form whose ultimate activity is dependent upon the types of substrates available at any one time. If we regard the proteins as the precursors, then their final enzymatic activity would depend on the available substrates and the genes would provide a *modus operandi* to allow it to proceed in the direction permitted by the existent conditions.

DR. BEADLE: Perhaps I should put the question in a different way. Is it possible that in the self-duplication of genes in general there exists a common inactive form which can be transformed in many ways to make different genes?

DR. MULLER: All the evidence we have points to a very general "precursor" for genes. The most essential property of a gene is expressed in its capacity to produce an exact duplicate irrespective, within wide limits, of associated genes or environmental conditions.

DR. SPIEGELMAN: There are several aspects of the pepsinogen transformation as an analogy that I would like to mention. The formation of pepsin from pepsinogen is spontaneous if pepsin and hydrogen ions are available, i. e., no energy is required. This is not true for the enzymes we are considering. Removal of the source of energy supply leads to cessation of enzyme formation. As far as the genic aspects of the problem are concerned, it seems to me little is gained by

the analogy. Pepsinogen already contains inherently all the specificity of pepsin. Thus the analogy would say that what we are observing in the kinetics of adaptation is a simple autocatalytic activation of inactive galactozymase or melibiozymase. Presumably the main function of the gene is specificity determination of the enzyme it controls. We would thus leave unexplained how the inactive forms of the enzymes with their inherent specificities could be synthesized in the absence of their specific genes.

DR. LINDEGREN: Isn't it true that a direct application of this analogy would mean that there was always available a considerable amount of this specific precursor even in the unadapted cell? This does not seem to be a reasonable situation.

DR. SPIEGELMAN: In this connection we might mention that when one tries to induce two enzymes simultaneously a competitive interaction can be established. This could be interpreted as competition for a common substrate which goes into the formation of the two different enzymes and would argue against any great specificity of the precursor.

DR. TATUM: It could be a competition for a limited source of energy.

DR. SPIEGELMAN: That is certainly true.

DR. HERSHEY: And it might be noted that shifting the gene action to the reaction forming "P" does not change the nature of the question.

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FOLLOWING READING OF HOLLAENDER'S PAPER

DR. RAPER: I might amplify just a bit what Dr. Hollaender was saying about this Aspergillus work. To test organisms for itaconic acid is time-consuming. Dr. Beadle does not know how fortunate he is in tracing down certain vitamins. Among the large number of cultures tested were about fourteen we thought particularly interesting. One culture did not increase the yield of total acid, but gave itaconic acid of exceptionally high purity. The other thirteen cultures did give total yields higher than the average for all of the controls which were run along with them, and of this number there were one or two that gave increases of 15 to 20 per cent. That is not a spectacular increase, but it is significant.

At the same time we have been isolating strains from nature. About 330 or so of these have been tested, and we now have a dozen or more that are better than our original stock. This merely goes to show that we did not have the best organisms to start with.

Dr. Hollaender alluded to the fact that they came from Texas. The majority have come from soils obtained from Texas, Arizona, New Mexico, and whether it means anything or not, Calcutta, India, and Western Australia. The temperatures are fairly high and the climate rather dry, a fact that may be of interest to you.

I would like to ask Dr. Hollaender whether the penicillin production tests

were run in submerged culture.

DR. HOLLAENDER: Yes.

DR. RAPER: Assays showed maximum yields at three days?

DR. HOLLAENDER: Yes.

DR. RAPER: Maximum yields at three days are a little unusual in the type of equipment with which we work. I do not know whether it is Dr. Beadle's experience or not.

DR. BEADLE: We would agree with you that they would come later.

DR. RAPER: I would raise one slight question about some of the yields. On the second-, third-, and fourth-day assays you would not expect the tremendous jump and then the abrupt fall shown in some of these figures. They jump from 18 to 60 to 20 on the second, third and fourth days respectively. One would expect that the slopes of that curve would be more gradual as a general thing.

DR. HOLLAENDER: We have data on five-day cultures that show fairly high yields but not so high as the three-day cultures.

DR. MULLER: If we assume that a given product, such as penicillin, has some adaptive value to the organism, and if we could find out the conditions under which it would help the organism, then by increasing the intensity of those conditions it might be possible to establish some sort of automatic selection. For example, if the penicillin helps to protect the Penicillium against competing bacteria, the addition of bacteria to a lot of cultures might produce such a condition.

DR. GODDARD: That was proposed some time ago and has been tried out in our laboratory and other laboratories, but we do not know of an actual stimulation or increase in productivity.

FOLLOWING READING OF GREENSTEIN'S PAPER

DR. STEINBACH: It is a rather interesting point that, as you say, you get the interaction between the nucleic acid and the globular proteins. The general tendency is to think of the protein chromosome matrix as more or less fibrous.

DR. GREENSTEIN: Since fibrous protein may at the same time be rather rolled the difference between fibrous and globular proteins may not be critical.

DR. STEINBACH: Is this interaction of certain nature? For example, if you are building up a chromosome is the nucleic acid a part of a definite pattern, stuck on the sides, or what would it be?

DR. GREENSTEIN: It is fixed only at fixed moments. The chromosome is not a stationary object; one must assume that it is changing continuously and one could expect that the physical properties of each of the components would be changed at each moment.

DR. SPIEGELMAN: What groups fix? Where does the interaction take place between the protein and the nucleic acid?

DR. GREENSTEIN: It must take place between charged groups, and this can have a marked effect upon the shape of nucleic acid.

DR. STURTEVANT: How combinations occur is of interest to geneticists who study specificity at different levels. Now, frequently it has been customary to think of genetic specificity as due largely to the protein. I take it that the current tendency is perhaps to ascribe it to the nucleic acid component. I wonder if you would be willing to throw some light on that question.

DR. GREENSTEIN: Nucleic acid, although of relatively simple structure, incapable of such alterations as proteins are, nevertheless can attach itself to certain types of proteins and produce an over-all difference in specificity. I don't quite see how a nucleic acid by itself can act; it must produce a combined effect. I think we are both coming to the conclusion that nucleic acid perhaps exerts its effect by contributing to reacting systems.

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FOLLOWING READING OF SONNEBORN'S PAPER

DR. SPIEGELMAN: I should like to suggest, Dr. Sonneborn, that the possibility of self-duplication of kappa is not entirely ruled out. Your conclusion depends on the fact that without the K gene the kappa disappears, but if we should assume that the precursor is always limiting, the amount of kappa that could be formed at any given time by self-duplication would be small. The velocity going from precursor to kappa would then be gene-controlled and you would always need the gene there to maintain kappa.

DR. SONNEBORN: Would you suggest any method by which it could be detected?

DR. SPIEGELMAN: Well, unless you know the precursor you cannot test it; that is true.

FOLLOWING READING OF GOWEN'S PAPER

DR. DELBRÜCK: You cited one instance of a spontaneous mutation, rate 1 in 180, and you determined the presence of the mutation by looking at the colony?

DR. GOWEN: Taking one of these micro-colonies and then plating out 100,000 cell groups on the assumption that you would have equal rate between the mutant type and the original type.

DR. DELBRÜCK: But you determined the character, smooth or rough, after your bacteria had grown into a colony?

DR. GOWEN: That is right.

DR. DELBRÜCK: How do you know the mutation does not occur in the colony?

DR. GOWEN: I don't. I expect it does occur in the colony.

DR. DELBRÜCK: Perhaps I misunderstood the procedure: Take one bacterium, let it grow into very small colonies; then pick out and plate individuals from the small colonies, letting each individual grow into a colony, and now you determine

whether these latter colonies are rough or smooth?

DR. GOWEN: Yes.

DR. DELBRÜCK: If the mutation rate is anywhere near 1 in 180 then all the colonies must be sectorial colonies because in each colony you grow individuals up to many millions.

DR. GOWEN: Rough and mixed, and some smooth, we think.

DR. DELBRÜCK: A colony consisting of one million smooth bacteria if your mutation rate is 1 in 180?

DR. GOWEN: I said we think they are smooth. If your mutation was toward the end of the process of course you would not be able to distinguish it.

QUESTION: How many cell generations do you have?

DR. GOWEN: About thirty minutes is the generation time.

DR. SPIEGELMAN: The experience I had some time ago with S. *aertrycke* bears out Dr. Delbrück's contention: we had a rapidly varying strain where rough gives off rough and smooth; when the mutation to the rough was high we got all rough.

DR. GOWEN: You were sure you started with rough colonies?

DR. SPIEGELMAN: Yes.

DR. GOWEN: We have not found many of those. In fact, offhand, I do not think I know of any with that particular set of characters.

DR. MULLER: It seems to me that the method will work if you have enough colonies, if you allow for the number of generations, and assume symmetrical reproduction. Can't you work it that way if you do enough colonies?

DR. GOWEN: That is right. Don't think for a minute that I think this technique is as good as micro-dissection technique, but after you work with the microscope for a year you also turn to other check methods as well.

DR. DELBRÜCK: I don't see how the micro-dissection technique can be of any help in determining mutants only recognizable in the character of the colony.

DR. GOWEN: If you grow the colony large enough, of course, you will get rough and smooth out of it and, a point I did not bring out, this change occurs in one cell generation.

DR. STURTEVANT: Perhaps I missed something. I would like to know how you keep this mutant stock in the first place.

DR. GOWEN: It is only through very rapid transfer that you may keep it. If you leave it any length of time it becomes rough very promptly. It is possible to classify the original micropipette-selected bacterium as either of the smooth type or the rough type in genetic constitution, because the mutant form leading to rough colonies is stable. All the bacteria coming from the single micropipette-isolated bacterium which is itself rough will themselves give only rough colonies. On the other hand, the bacterium forming a smooth colony is unstable. Samples taken from a colony originating from such a smooth-type bacterium will show colonies which are smooth, smooth and rough sectored, and rough.

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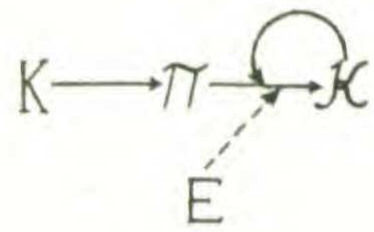
FINAL DISCUSSION

DR. MULLER: Dr. Lindegren has asked me to give a more detailed picture of the hypothesis that I suggested yesterday, when I attempted to bring his and Dr. Sonneborn's findings under the same scheme. For this purpose let us consider the diagram here shown:



Here, at the beginning, you have Dr. Sonneborn's "Killer gene", a large "K", and this produces a certain gene product, π, which in turn is required for the production of the cytoplasmic substance kappa, κ. The latter, in our present scheme, is to be considered as corresponding with the "cytogene" of Lindegren. For reasons that will appear, it seems likely that the product π is closely related in its composition to the gene K that produced it, and also to the substance kappa, or "cytogene", that follows it. If so, π may be regarded as the precursor of kappa, and would represent the intermediate step in at least two successive modifications of K. It is not absolutely necessary for the scheme, however, to assume these relationships in composition of the three substances concerned.

Now, the presence of π would not by itself be sufficient, in either Lindegren's or Sonneborn's cases, for the production of kappa. But if some kappa is already present to begin with, the reaction $\pi \rightarrow \kappa$ is activated by this kappa, as indicated in the diagram by the curved arrow that arcs upwards and backwards from kappa and impinges on the arrow leading from π to κ. Thus more kappa, or cytogene, becomes formed. If π and κ are related in composition, this reaction might be compared (as has been done by others independently) to the effect of pepsin in transforming pepsinogen into more pepsin, although it is not yet known whether this type of self-activation is common for enzymes. However, on the assumption that K, π and κ are all related, this self-converting effect of kappa could be regarded as due to kappa having preserved in itself something of the nature of a gene. It would not have a gene's more generalized ability of converting non-specific materials of the medium into material like itself, but it would have a more limited ability of thus converting a specific precursor, one which had already gone a large part of the way in the shaping of the final material.

Now, if the gene K should be removed, the chain of reactions is broken, and no more kappa can be produced. This breakage has occurred in Sonneborn's material, by mutation of the gene K to k, but nothing analogous to this mutation has yet been found in Lindegren's material. Removal of kappa also, in Sonneborn's material, breaks the reaction chain, so as to stop further kappa production. But this is not always true of what Lindegren has called the cytogene in his material. In his case, even though this cytogene is in some lines necessary for the production

of more material of its own kind, in other lines, differing from the former in a single pair of genes, which we may here designate as E versus e, the final substance or cytogene can be produced even when there is none of it present to begin with. The gene E of yeast then, or a product of it, must here be able, like kappa itself, to activate the reaction $\pi \rightarrow \kappa$ so that this reaction will go on even in the absence of initial κ. If kappa (here the cytogene) were closely related to π in composition, it would not be surprising that the conversion could be effected by other means than by kappa itself, as is true also of the pepsinogen-pepsin conversion. (The alternative scheme, not relating the two sets of results, would tend to have the cytogene similar to E instead. In the *Paramecium* material, it is as though none of the lines discovered contain E, but only e (or the absence of any comparable gene).

Another difference in the two materials lies in the fact that, for persistence of the cytogene, but not, so far as known, for that of the kappa of *Paramecia,* a certain substrate, melibiose, is required. If the cytogene can be identified with the enzyme melibiose-zymase which enables us to recognize its presence, this requirement is quite understandable, as many enzymes deteriorate rapidly in the absence of their specific substrates. But this very requirement helps us to understand also the presence of E in the yeast material, since without E the cytogene would be permanently lost every time a line of cells happened to grow in a medium lacking this specific substrate. In *Paramecia,* where there seems to be no such specific external substances which are likely to be missing from the environment, there is no need for such a gene as E to have become incorporated into the reaction system.

Of course the scheme here outlined is merely one conceivable possibility for relating the results on the two different organisms. The test of it would lie in the proof or disproof of the conception that the cytogene, like kappa, and unlike the genes of the chromosomes, continuously depends for its production on certain specific material (π), derived from or due to a particular gene.